

BioMag[®] SelectaPure[™] Anti-CD45 Antibody

Description:

CD45 a major component of the lymphocyte membrane is present on the surface of all human leucocytes, lymphocytes, eosinophils, monocytes, basophils and neutrophils. In addition, CD45 antibodies react with the leucocyte progenitors found in bone marrow. CD45 is lost during maturation of erythroid cells in the bone marrow and is absent from erythrocytes and platelets.

The group of 5 CD45 isoforms are single chain integral membrane proteins, ranging from 180 to 220 kDa. The different isoforms are generated by alternative splicing of three exons of the genomic sequence. The Leucocyte Common Antigen consists of an extracellular proximal membrane sequence common to all CD45 isoforms. All the monoclonal antibodies of the CD45 cluster react with this part of the antigen and thus are able to recognize all of the CD45 isoforms. The different isoforms have extracytoplasmic sequences ranging from 391 to 552 amino acids which have numerous N-linked carbohydrate attachment sites. The cytoplasmic portion of CD45 contains two phospho-tyrosine-phosphatase domains.

BioMag Anti-CD45 particles recognize the leukocyte common antigen and are used for bone marrow and peripheral blood depletion of malignant and normal lymphocytes, including B cells and granulocytes. BioMag Anti-CD45 particles may also be useful in studies involving T cell activation and inhibition.

Particle Concentration

The concentration of BioMag is approximately 4 to 5 mg/ml. There are approximately 1×10^8 BioMag particles per mg.

Cell Separation Recommendations

Depending upon antigen availability and the size of the target cell population, cell sorting applications may require up to 50-60 magnetic particles per cell based on the target cell population. Magnetic particles and cells should be incubated at room temperature for 30 minutes to one hour in media containing 5-10% protein (to reduce non-specific binding) for successful separation. Gentle end over end or rocking during incubation is required for optimal results. (Note: Increasing

the incubation time beyond one hour may be necessary to achieve the desired depletion.) Each researcher must optimize particle to cell ratio and incubation time for the application.

Some applications require the detachment of BioMag antibody particles from cells after separation. One approach would involve culturing cells after positive selection. Cultures can be maintained for about 48 hours during which magnetic particles fall away from cells due to cell surface changeover. The magnetic particles are then easily removed via a magnetic separation. Another approach is the use of a protease such as chymopapain to break the antigen-antibody bond and remove the particles magnetically. Depending upon the application, it may not be necessary to remove the cells from the BioMag particles. BioMag particles are only 1 μ m in size and have been successfully used in FACS equipment. They will not jam the machine and are distinguishable from cells. Alternatively, negative selection approaches can be very effective in producing specific cell populations.

Storage and Stability

The suspension is supplied in PBS/EDTA/1.0% BSA/0.1% sodium azide buffer at pH 7.5. Washing BioMag Anti-CD45 particles in sterile media to remove preservative prior to use is recommended. Using a magnetic separation unit for washing instead of centrifugation is strongly recommended. Do not freeze, dry or centrifuge BioMag. Freezing, drying and centrifuging BioMag can result in aggregation and loss of binding activity. BioMag Anti-CD45 particles are stable when stored at 4°C.

Safety

BioMag Anti-CD45 particle suspension contains sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal of material, flush with a large volume of water to prevent azide accumulation. Please consult the Material Safety Data Sheet for more information.

Ordering Information:

Figure A-1

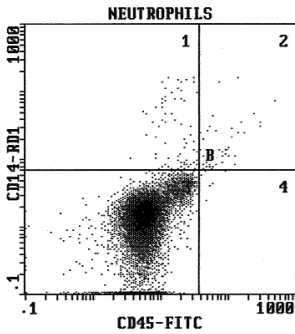


Figure A-2

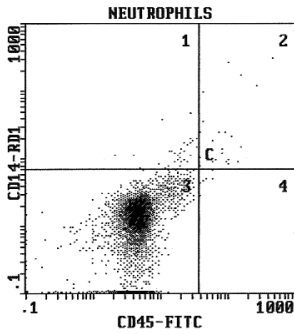


Figure A-3

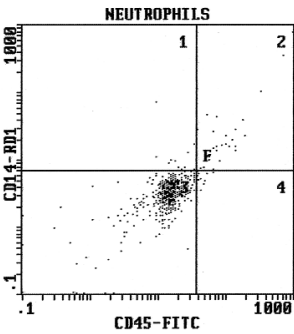


Figure B-1

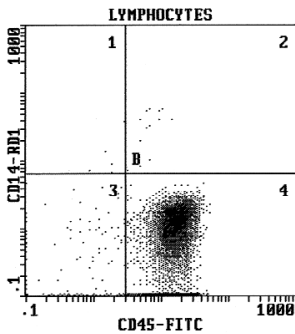


Figure B-2

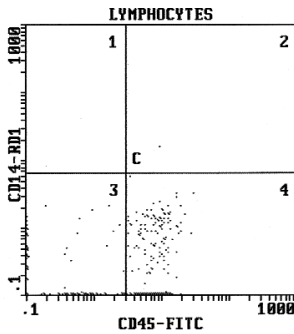


Figure B-3

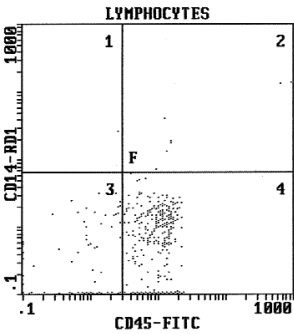


Figure C-1

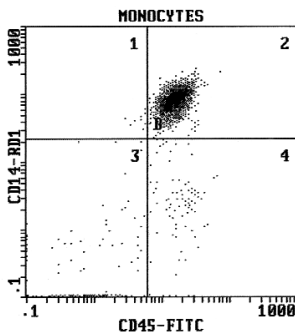


Figure C-2

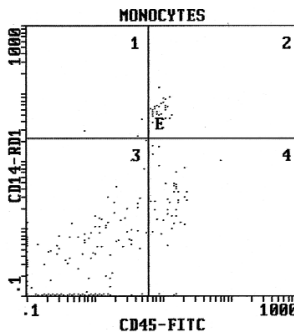
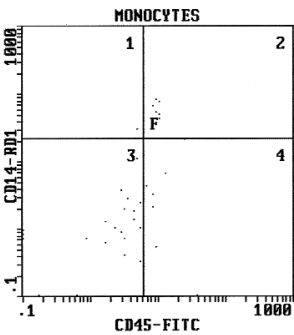


Figure C-3



Cell sorting results using BioMag Selectapture CD45 anti-human leukocyte particles for positive selection. Typically whole blood or purified leukocytes and particles are incubated for 30 minutes at room temperature and then magnetically separated. The supernatant is collected, incubated with the appropriate two-color antibody cocktail, and then analyzed by flow cytometry. Figure A1 depicts the neutrophil population prior to positive selection. A2 shows the cell population after positive selection with BioMag anti CD45 particles. A3 shows the cell population after positive selection with CD45 and CD16 combined in equal ratios. B1 - B3 depicts the results for lymphocytes and C1-C3 depicts the results for monocytes. The particle to cell ratios reported to the left are based on experiments where cells were exposed to the particles once.

General Recommendation - BioMag CD45 for Lymphocytes*:

Conc. #	4.00 x 10 ⁸ bead/ml
Vol. Used	0.200 ml
# Particles	8.00 x 10 ⁷ per test
# Target Cells	1.72 x 10 ⁶ per test
Bead:Cell Ratio	46.5
% Depletion	97.36%

General Recommendation - BioMag CD45 & 16 for Lymphocytes*:

Conc. #	4.00 x 10 ⁸ bead/ml
Vol. Used	0.100 ml (50 ml each)
# Particles	4.00 x 10 ⁷ per test
# Target Cells	1.72 x 10 ⁶ per test
Bead:Cell Ratio	23.3
% Depletion	96.73%

*These values should be used as a starting point in optimizing experimental protocols. Due to differences in the distribution of cell types in samples and other variables, the researcher is strongly encouraged to determine the optimal particle to cell ratios for their experiments.

Cat. #	Description	Size
85045	BioMag Anti-CD45 Antibody	1 ml 5 ml

To Order:

In The U.S. Call: 1-800-523-2575 • 215-343-6484
In The U.S. FAX: 1-800-343-3291 • 215-343-0214

In Germany Call: (49) 6221-765767
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